

Using Lanes

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Using Lanes

Lane analysis is separated into 2D analysis and Profile (1D) analysis. This chapter discusses 2D analysis. For a discussion of Profile (1D) analysis, see the chapter *Profile (1D) Analysis*.

Samples organized in lanes or tracks can be analyzed with a lane template. Examples include one dimensional electrophoresis or thin layer chromatography samples. Each template can be comprised of one or many lanes. Using lanes, you can quantitate the activity in bands or spots and determine molecular weights Rf values.

Templates include customized report options. These templates can be saved for use with other samples.

Creating a Lane Template

The **Lane Template Parameters** window (Figure 7-1) enables you to define a template of lanes.

To display the **Lane Template Parameters** window:

- ☐ Select **Create Template** from the **Analysis** menu.

Or,

- ☐ Select **EDIT** from the template bar.

The **Create Template** window is displayed.

- ☐ Select "Lanes" at the **Create Template** window.
- ☐ Select **OK**.

The selections at the **Lane Template Parameters** window are described below.

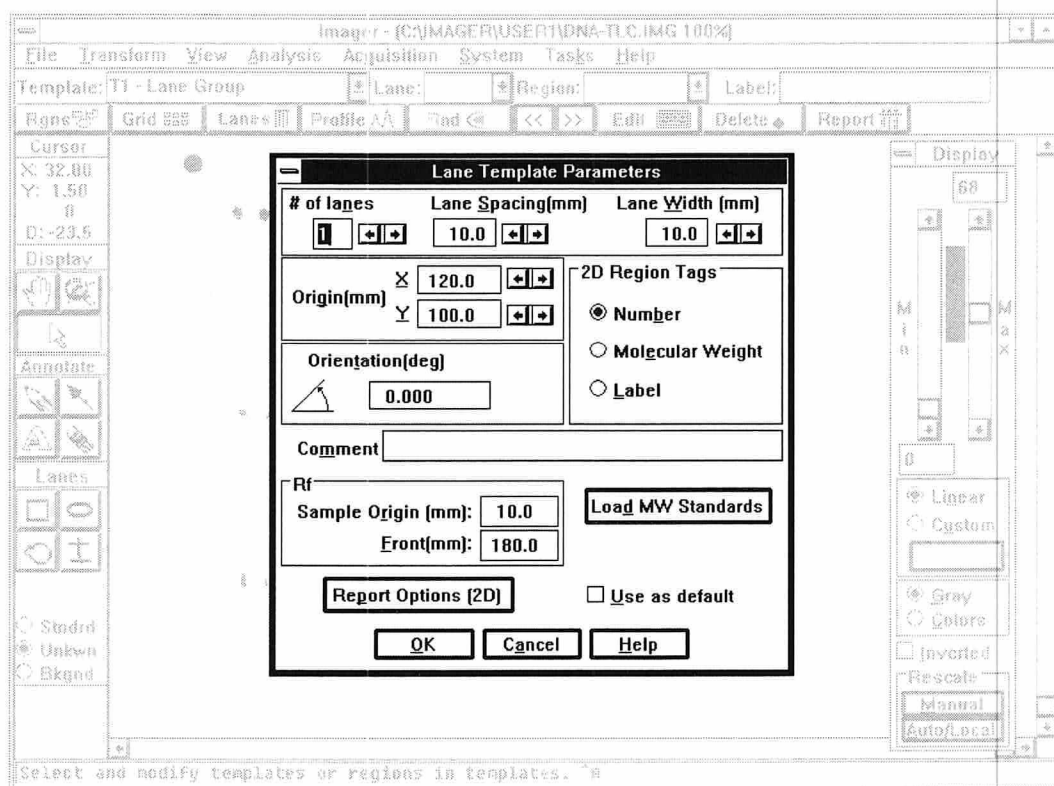


Figure 7-1. Lane Template Parameters Window.

Select **OK** after you have responded to the selections described below. Once you do, a template tag (like "T1") is displayed to the right of the template (upper right corner). This tag identifies the template. Click on this tag to select the template.

Each lane is identified along the line of origin. Click on the lane tag to select the lane.

To create a second lane template, revisit the **Lane Template Parameters** window and select **OK**. The next available template tag ("T2") is displayed to the right of the template. This tag identifies the second template.

An example lane template is shown in Figure 7-2.

SELECTION

DESCRIPTION OF USE

of Lanes

Specify the number of lanes in the template. The maximum number is 50.

Lane Spacing (mm)

Specify the spacing of the lanes (the spacing is measured in millimeters).

Profile Width (mm)

Specify the width of lanes for use in profiles (in millimeters). Make the width of the profiles smaller than the spacing of the lanes. If the profile width is greater than the lane spacing, it (the profile width) will be set to the value specified for the lane spacing.

All the lanes will be set to the profile width. However, the widths of the individual lanes can be changed by editing them on the screen (graphically).

SELECTION**DESCRIPTION OF USE****Origin (mm) X,Y**

Specify the X and Y coordinates of the outside edge of lane one. When creating a new template, these coordinates are near the center of the image as it appears on the screen.

Orientation (deg)

Adjust the orientation of the template if the sample is not placed in the instrument with a precise horizontal-vertical orientation.

**Region Tag:
Number**

Select this option to tag the regions with the region number. Regions are always numbered from the origin of the template.

**Region Tag:
Molecular Weight**

Select this option to tag the regions on the image with the molecular weight. If the molecular weight standards are not defined, the regions are identified with numbers.

**Region Tag:
Label**

Select this option to tag the regions on the image with user-defined labels.

Comment

Enter a comment that will be indicated when results are reported with the template.

**Rf:
Origin (mm)**

Specify the distance (in millimeters) from the top of the template (line of origin) to the sample origin (within the template).

**Rf:
Front (mm)**

Specify the distance (in millimeters) from the top of the template to the solvent front (within the template).

SELECTION

Use as Default

DESCRIPTION OF USE

Select the check box if you want to use the current parameters every time you create a template with this user. Graphically sized lane lengths are also saved with the default.

LOAD MW STANDARDS

Displays the **Load Standards File** window.

REPORT OPTIONS

Displays the **Lane Group Report Options** window.

OK

Saves the parameters and creates the template. The template becomes the selected object on the image.

CANCEL

Aborts the operation. Closes the window.

HELP

Displays online documentation that describes the function of the current window.

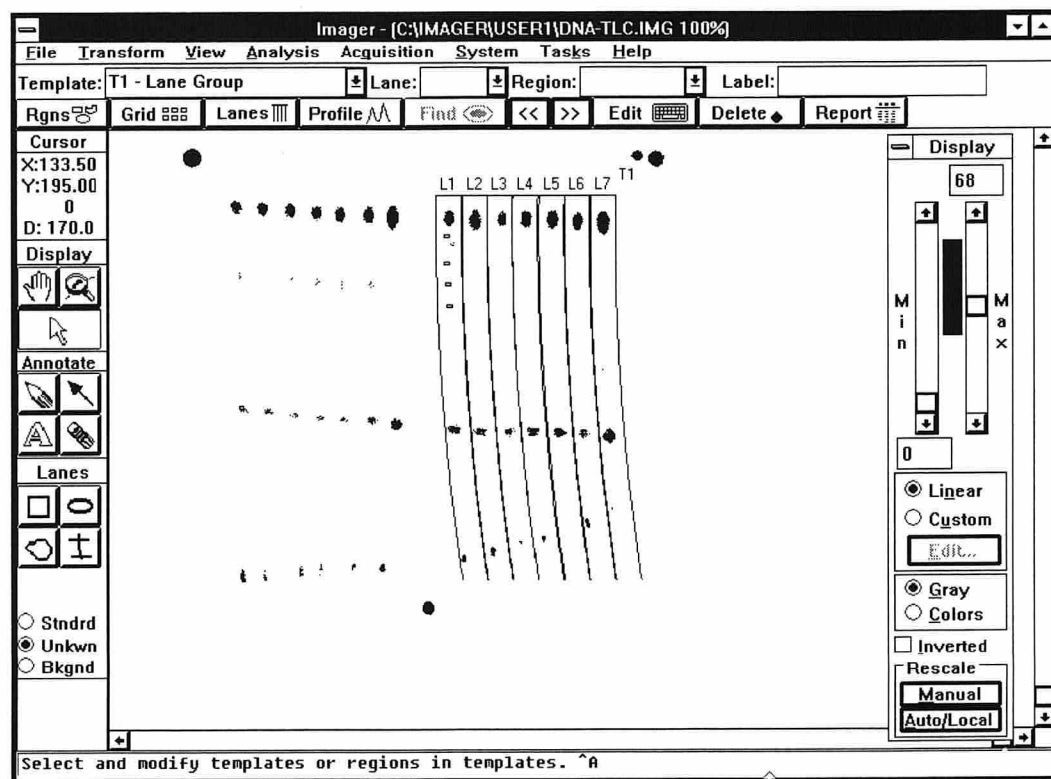


Figure 7-2. Lane Template.

Editing Lane Templates Graphically

Once created, lane templates can be edited on the screen to fit the template to the image. There are handles on the template which can activate editing functions when you move the pointer over them.

You can edit an entire template or an individual lane within a template. You must use the pointer to edit a template:

☐ Select  in the tool box.

Next you must select the template.

See the section *Selecting Objects in Lane Templates* (page R248).

Moving and Copying the Template

When a template is selected, the pointer will become a move/copy cursor when it is moved inside the template.

The move/copy cursor looks like this: 

The template can be dragged using the left mouse button.

If you press the <Ctrl> key while you press the left mouse button (or if you use only the right mouse button), the entire template will be copied to where you drag the mouse.

Resizing Width and Height

The pointer will become a resizing cursor when moved over the two square resizing handles at each end of the origin line of the template.

The resizing cursor looks like this: 

You can then drag the handles with the left mouse button to change the origin line. The center of the lane opposite the resizing handle that you are moving remains fixed.

Resize the Length

The pointer will become a resizing cursor over the single square resizing handle located at the center of template opposite the origin.

Drag the end of the template with the left mouse button to change the length.

The length of the template will be indicated in the tool box (cursor box) as you drag with the mouse.

►► **Tip:** *To change the default length for new lane templates that you create, resize the lane length as described above and then access the **Lane Template Parameters** dialog box by clicking the **EDIT** button. Make sure that all of the template parameters are defined as desired and mark the "Use as Default" check box before you close the dialog by clicking on the **OK** button. Defaults are saved separately for each user.*

Rotate the Template

The pointer will become a rotation cursor over the rotation handle. The rotation handle is at the center of the last lane (farthest to the right).

The rotation cursor looks like this: 

The center of rotation is located at the center of lane one.

Press the left mouse button and while holding it down, move the mouse off the rotation handle. Rotate the template to the desired location. When the mouse button is released, the template will be redrawn in the new orientation.

Editing Individual Lanes Graphically

If you plan to specify regions by integrating the area under peaks in profiles of the lanes, it may be necessary to edit the width, position, or curvature of individual lanes to obtain reliable results.

If you plan to place two dimensional regions around bands or spots, or only mark their position, it will seldom be necessary to edit individual lanes. In this case the lanes serve to identify the regions with the samples and to provide a coordinate system to determine migration distances.

Two dimensional regions are assigned automatically to lanes according to the position of the centroid of the counts, and unless the lanes or tracks are strongly curved, correct assignments will be made with straight template lanes.

You must use the pointer to graphically edit an individual lane in a template:

☐ Select  in the tool box.

Next, select the lane. See the section *Selecting Objects in Lane Templates* (page R248).

Moving the Lane

The pointer will become a move cursor when moved over the selected lane near the origin.

The move cursor looks like this: 

If the lane profile width is less than the lane spacing, you can drag the lane along the origin line with the left mouse button.

Resizing the Lane Width

The pointer will become a resizing cursor when moved over two square resizing handles on the origin line at each edge of the selected lane.

The resizing cursor looks like this: 

Drag either edge of the lane along the origin line to change the profile width of the lane.

If you drag an edge of the lane into an adjacent lane, you will be able to push the edge of the adjacent lane until its width is decreased to 2 mm.

You can increase the size of the adjacent lane.

Curving the Lane

You can edit the curvature of one lane or all lanes within the template. Use the triangular handles to curve a lane. The mouse cursor becomes a lane curving cursor when you move the pointer over the triangular handles.

The handle located midway between the origin and the end of the lane can be dragged anywhere within the template with the left mouse button.

The handle located at the end of the lane can only be dragged along the end of the template.

As either of these handles is dragged, a quadratic curve is fit from the center of the lane at the origin line through the two lane curving handles. When the mouse button is released, the edges of the lane are redrawn on each side of this curve with a constant lane profile width.

You can edit the curvature of the selected lane by pressing the left mouse button. Use the **<Ctrl>** key with the left mouse button (or only the right mouse button) to edit the curvature of all lanes in the template simultaneously.

Selecting Objects in Lane Templates

You can select objects in lane templates with the pointer or in the drop-down menus in the template bar.

To select a template:

- ☐ Move the pointer over the template tag (T1, T2, etc.). The pointer becomes a pointing finger.
- ☐ Select the template tag.

Or,

- ☐ Select the drop-down arrow in the template drop-down menu. The list drops down.
- ☐ Select the template from the list.

When the template is selected, the entire template and all the regions (if any) will be highlighted. The template is listed in the template drop-down menu (in the template bar). The lane and region drop-down menus will be empty, since no region or lane is selected. If a label has been created for the template, it will be listed in the label box in the template bar.

The template editing handles are also displayed; provided that no regions of interest are defined.

Two square resizing handles are displayed at each end of the origin line of the template. A single square resizing handle is located at the center of the template opposite the origin.

A round rotation handle is displayed at the center of the last lane (farthest to the right).

The pointer will become a resizing cursor when moved over the resizing handles. The pointer will become a rotation cursor when placed over the rotation handle at the center of the last lane (farthest to the right).

To select a lane:

- ☐ Move the pointer over the lane tag (L1, L2, etc.). The pointer becomes a pointing finger.
- ☐ Select the lane tag.

Or,

- ☐ Select the drop-down arrow in the lane drop-down menu. The list drops down.
- ☐ Select the lane number from the list.

When a lane is selected, the lane tag and the lane will be highlighted. The template and the selected lane will be listed in the appropriate template bar drop-down menus. If a label exists for the selected lane, it will be listed in the label box of the template bar.

Editing handles are displayed on the selected lane.

Two square resizing handles are located on the origin line at the edge of the selected lane. The pointer will become a resizing cursor when moved over resizing handles.

The triangular handles enable you to edit the curvature of one lane or all the lanes. See the section *Curving the Lane* (page R247).

Drawing Regions in Lanes

When regions are created in the template, the overall position, size, and orientation of the template become fixed; editing handles for the template are not present.

The region drawing tools will be displayed in the tool box when the lane template is created. The tools include:

-  The Rectangle tool enables you to draw rectangular regions.
-  The Ellipse tool enables you to draw elliptical regions.
-  The Irregular tool enables you to enclose an ROI with an arbitrary Shape.
-  The Band Marking tool enables you to mark the positions of bands in the lanes of a template. The regions will be automatically assigned to the lane in which they are drawn.

The Profile Drawing Tool is displayed in the tool box when a profile of the lanes is created:

-  The Profile Drawing Tool enables you to integrate the area under the profile within the profile display.

Single clicking and double clicking on a tool have different effects:

- ☐ Single click on a tool to select it for a single use, after which the pointer will be the selected tool.
- ☐ Double click on a tool for multiple uses, until another tool is selected from the tool box.

When moved over the image, the mouse cursor changes to indicate the function of the tool. For example, when you select a rectangle, a small square will appear next to the pointer.

Use the left mouse button to draw on the image.

The last region drawn will be the selected region and will be listed in the Region drop-down menu in the template bar. You can edit or delete the selected region by choosing **Edit** or **Delete** respectively from the **Analysis** menu.



Note: When drawing regions with the Rectangle tool, the Ellipse tool, Irregular tool, or Profile tool, the distance used in calculating the molecular weight is the centroid of the area covered.

Rectangle Drawing Tool

To draw a rectangular region within a lane:

- ☐ Select  from the tool box.
- ☐ Press the left mouse button to anchor one corner of the rectangle within the lane.
- ☐ Drag out the opposite corner of the rectangle with the mouse and release the mouse button.

Ellipse Drawing Tool

To draw an elliptical region within a lane:

- ☐ Select  from the tool box.
- ☐ Press the left mouse button to anchor one corner of the bounding rectangle of the ellipse within the lane.
- ☐ Drag out the ellipse and release the mouse button.

Irregular Drawing Tool

To draw an irregular region within a lane:

- ☐ Select  from the tool box.
- ☐ Press the left mouse button to begin drawing.
- ☐ Drag the mouse to enclose the ROI within the lane with an arbitrary shape.
- ☐ Draw the line to the starting point to close the irregular region.

If the border of the region crosses itself, the drawing process will stop, and the enclosed area will be the irregular region.

- ☐ Release and click the left mouse button to make a straight edge.
- ☐ Double click the left mouse button to close the irregular line with a straight line to the starting point.

Band Mark Tool

To mark a band in a lane within a lane:

- ☐ Select  from the tool box.
- ☐ Move the pointer to a spot in the selected lane.
- ☐ Press the left mouse button.

Profile Drawing Tool

You can draw profile regions in one lane at a time or in several lanes at once.

To draw profile regions in lanes:

1. Create profiles of the lanes of interest (see the section *Profiling Lanes*, page R256).
2. Select the lanes in which to draw regions:
 - Select a single lane.
 - Select the whole template to select all of the lanes.
3. Select  from the tool box.
4. Move the tool over the profile display lane and drag along the profile of the selected lanes.

A profile cursor line moves with the mouse. The cursor line is parallel to the template origin line. It passes through the profile display and all lanes that are profiled. The cursor box at the top of the tool box indicates the distance of the cursor from the origin line.

Qualifier Buttons

Below the region drawing tool icons in the tool box are qualifier buttons. These buttons determine whether the regions will be considered as unknowns (Unkwn), background (Bkgnd), or standards (Stndrd).

More than one region can be identified as a background region. The total counts in the background regions will be divided by the total area of the regions to determine the background.

To define the region as a background:

- ☐ Select "Bkgnd" in the tool box.

To define the region as an unknown:

- ☐ Select "Unkwn" in the tool box. The qualifier becomes "Unkwn" after a region is defined.

To define the region as a standard:

- ☐ Select "Stndrd" in the tool box. A window listing molecular weight values will appear.

After the qualifier has been selected, you can create the region. Note that regions defined as standards must have molecular weights assigned to them; see the section *Determining Molecular Weights*, page R257).

Editing Regions in Lanes

Regions of interest can be specified in lanes by drawing two dimensional regions or by integrating the area under profiles.

When a region is selected, handles are present which can activate the editing functions when you move the pointer tool over them. The editing handles for two dimensional regions are on the regions on the lanes(s). The editing handles for regions under the profile are on the profile display.

Selecting a Region

To select a region in a lane:

- ☐ Move the pointer over the lane tag (L1, L2, etc.). The pointer becomes a pointing finger. Click on the tag.
- ☐ Move the pointer over the region tag (1,2, etc.). Click on the tag.

Moving or Copying the Region

When you move the pointer over a selected region, the mouse cursor becomes a move cursor.

The move cursor looks like this: 

Press the left mouse button and drag the region to a new location. The region is redrawn when you release the left mouse button.

If you drag the region with the **<Ctrl>** key while you press the left mouse button, the pointer becomes a copy cursor. The region is copied when you release the **<Ctrl>** key and left mouse button.

Resizing the Region

A selected region or ellipse will have resizing handles. The pointer becomes a resizing cursor over the resizing handles on the rectangle or ellipse.

The resizing cursor looks like this: 

You can drag the edges or corners of the regions with the left mouse button.

Deleting a Region

A region can be deleted from a lane:

- ☐ Select the lane and region (see *Selecting a Region*, page R254).
- ☐ Select **Delete Template** from the **Analysis** menu.

Or,

- ☐ Select **DELETE** from the template bar.

Labeling Lanes and Regions

You can assign labels to lanes or regions within a lane. You can also choose to print the labels on the image and in reports.

A label for a selected region can be added or edited in the template bar.

To enter labels as you draw regions:

- ☐ Draw a region, which will become the selected region. Then select the Label box in the template bar and enter a label into the box.
- ☐ Draw a second region and enter a label for that region from the keyboard. (The Label box will have the focus from the first time you clicked on it, and the text you enter will appear in the box.)

Profile Analysis

After a lane template is overlaid on an image, you can click on the Profile button (or select Lane Profile for the Analysis menu) to create profiles of the lanes. The profiles will be displayed in a special window. You must select a lane template before a profile can be created. (See the section *Selecting Objects in Lane Templates*, page R248).

For more information on profile analysis, see the chapter *Profile (1D) Analysis* in this manual.

Adjusting the Template

The template you select can contain one or more lanes. It must be placed over the lanes of the sample (image). If the sample has a format that you have used before and you load a template to fit that format, you may have to adjust the position and orientation of the template. Likewise, a newly created template also must be adjusted.

To adjust a template to fit the orientation of the sample:

1. Move the overall template to place the origin of the template at the edge origin of the gel or TLC plate. Place the center of lane 1 at the center of the first lane of the sample.
2. Drag the resizing handles at the end of the origin line to match the line spacing of the sample.
3. Drag the resizing handle at the end of the template to the required lane length.
4. Rotate the template if necessary using the rotation handle. This is necessary if the sample was not placed squarely on the sample tray.
5. If you want the profile to be a slice from the center of each lane, adjust the profile width in the **Lane Template Parameters** window (Figure 7-1). The profile width is always set to the lane spacing if the width of the template is edited graphically.
6. Adjust the orientation of the individual lanes if the lanes are non-uniform or curved.

See the sections *Editing Lane Templates Graphically* (page R244) and *Editing Individual Lanes Graphically* (page R246) for more information on how to adjust the template.

Determining Molecular Weights

Molecular weights (MWs) of unknown regions or bands are determined automatically when MW values are assigned to bands designated as standards.

You must use the **Mark Standard** (Figure 7-3) window to designate regions as standards and assign MWs to them. The **Mark Standard** window is displayed when you select the “Stdndr” qualifier in the tool box. Close the window after marking standards by selecting either the “Unkwn” or “Bkgnd” qualifier.

When the window is opened, the default standards list is displayed. The first standard in the list is highlighted. The list also contains comments attached to the standards when the list was created.

To determine molecular weights in a lane:

- ☐ Select the **Stdndr** button in the tool box. (A lane template must be displayed). The **Mark Standard** window is displayed.
- ☐ Select a lane containing MW standards samples (see the section *Selecting Objects in Lane Templates*, page R248).
- ☐ Select a drawing tool (e.g., the Band Marking tool). See the section *Drawing Regions in Lanes*, page R250).
- ☐ Mark a point in the lane with the Band Marking tool or create a region in a lane. The highlighted standard in the list is assigned to the point.

The next highlighted MW standard in the list is assigned to the next band marked or region created.

You can select any standard in the list by clicking on it with the left mouse button.

- ☐ Continue to create the regions in the lane using the **Mark Standard** window.

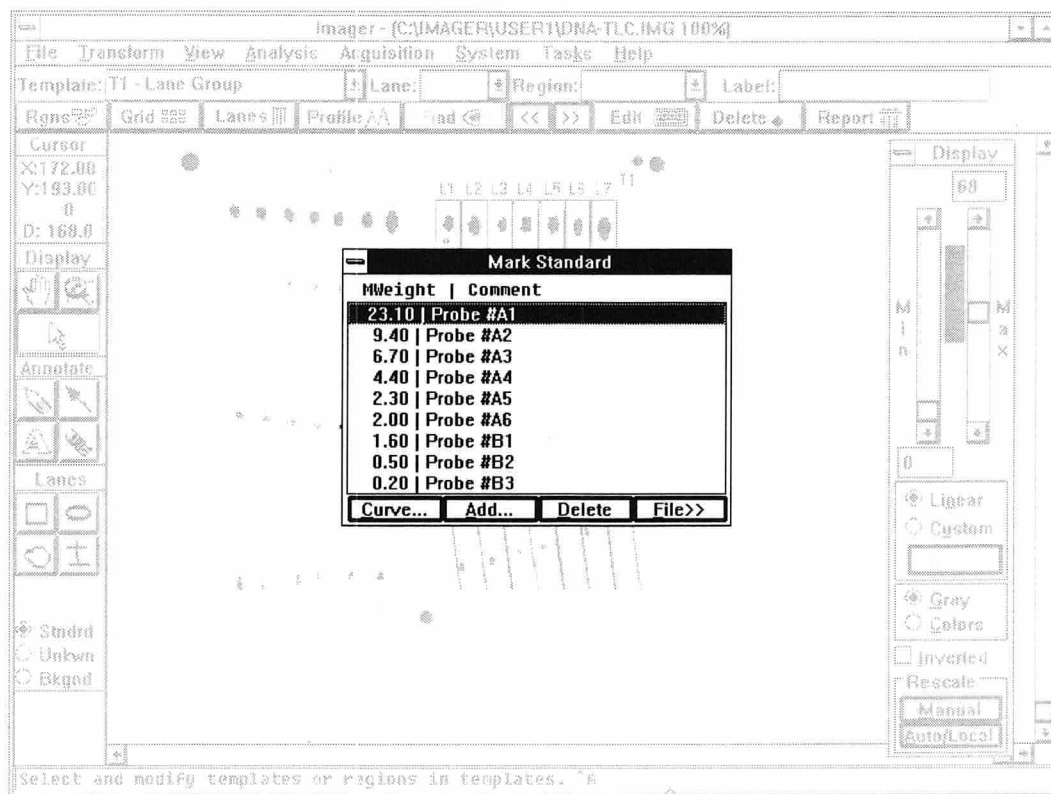


Figure 7-3. Mark Standard Window.

Deleting a Standard From the List

Any standards added and displayed at the **Mark Standard** window can be deleted.

To delete a standard:

- ☐ Select a standard in the list.
- ☐ Select **DELETE**.

Adding Standards to the List

You can add standards to the list at the **Mark Standard** window. A molecular weight and comment can be assigned to each standard that you add.

As you add standards, the list will be automatically sorted from the largest MWs to the smallest MWs.

The **Add Standard to List** (Figure 7-4) window enables you to add standards to the list in the **Mark Standard** window.

To display the **Add Standard to List** window:

- ☐ Select **ADD** at the **Mark Standard** window.

Select **ADD** or press **<Enter>** after each time you enter a weight (and a comment if desired).

Select **CANCEL** when you are done.

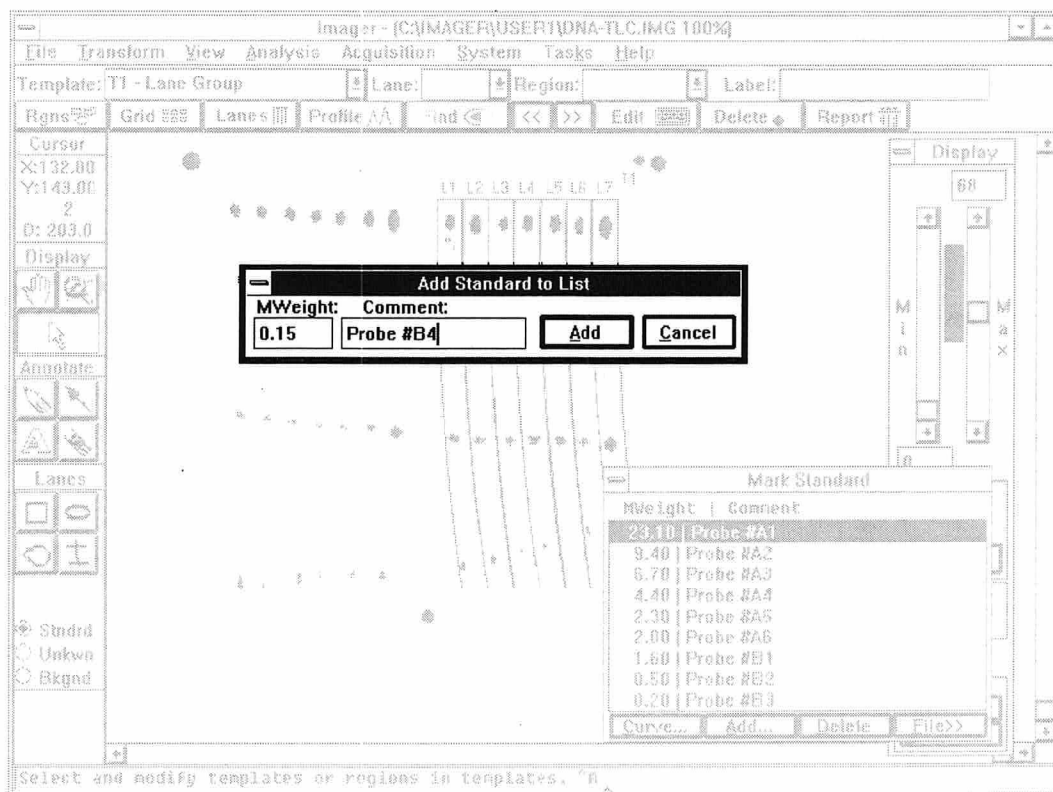


Figure 7-4. Add Standard to List Window.

SELECTION

DESCRIPTION OF USE

MWeight:

Enter the molecular weight of the standard.

Comment:

Enter a brief comment that identifies the standard.

ADD

Adds the standard (molecular weight and comment) to the list. Select by pressing **<Enter>**.

CANCEL

Terminates the operation and closes the window.

Molecular Weight Standard Curve

The standard curve represents the relationship between distance and the molecular weight for the standards assigned in the lane. Distance is measured from the origin line to the standard. The system uses this curve to determine the molecular weights of unknowns.

You can display the standard curve at the **Molecular Weight Standard Curve** window (Figure 7-5).

To display the **Molecular Weight Standard Curve** window:

- ☐ Select **CURVE** at the **Mark Standard** window. ("Stndrd" must be selected in the tool box.)

Note that the curve cannot be printed.

Selections at the window are discussed below.

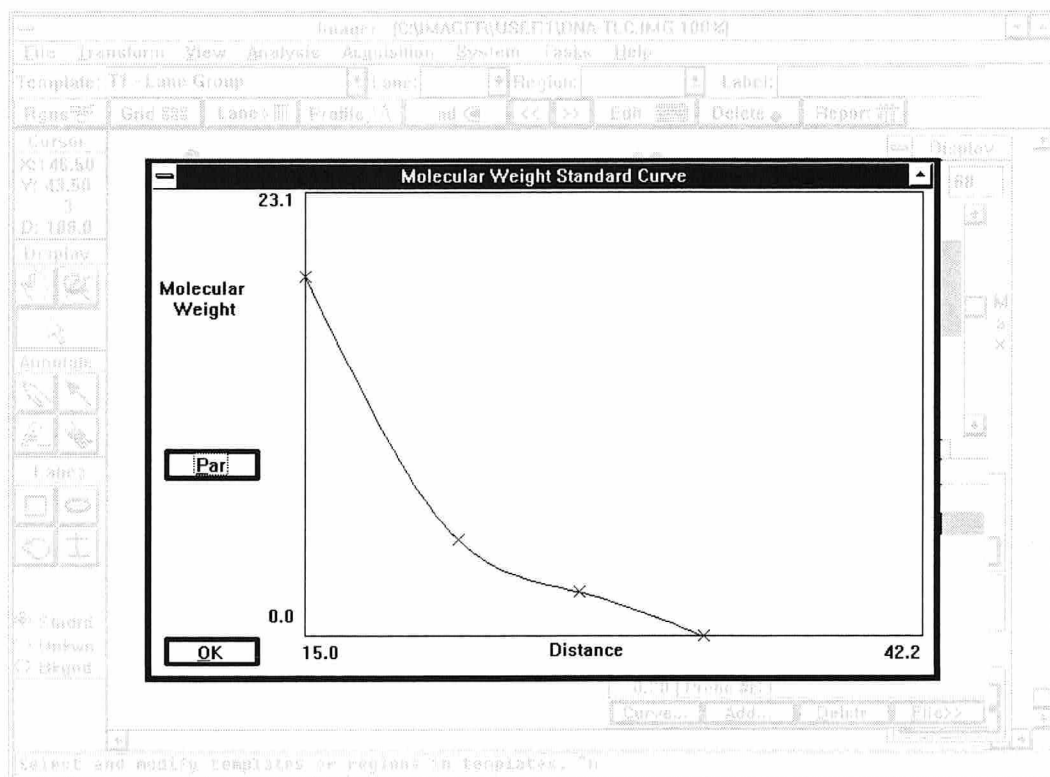


Figure 7-5. Molecular Weight Standard Curve Window.

SELECTION

DESCRIPTION OF USE

PAR

Displays the **Standard Curve Parameters** window (Figure 7-6). This window enables you to select the curve fitting method and the axis transformation for the standard curve. See the section *Selecting Standard Curve Parameters*, page R262).

OK

Closes the window.

Selecting Standard Curve Parameters

You can select the appropriate curve fitting method and axis transformation for the standard curve displayed at the **Molecular Weight Standard Curve** window.

The **Standard Curve Parameters** window (Figure 7-6) enables you to make these selections. Once the selections are made, the curve is automatically redrawn for you on the screen. This tool enables you to find the most suitable curve.

To display the **Standard Curve Parameters** window:

- ☐ Select **PAR** at the **Molecular Weight Standard Curve** window.

Each selection at the window is described below. Selecting **OK** draws the standard curve based upon your transformation selection and curve fit selection.

Note that MW vs Distance is the default transformation setting and that Spline is the default curve setting.

 **Note:** *The calculations used in the instrument are based upon standard mathematical formulas. See the following resources for more information: 1) "Numerical Methods" by Robert W. Hornbeck – 1975. 2) "Smoothing By Spline Functions" by Christian Reinsch – 1967.*

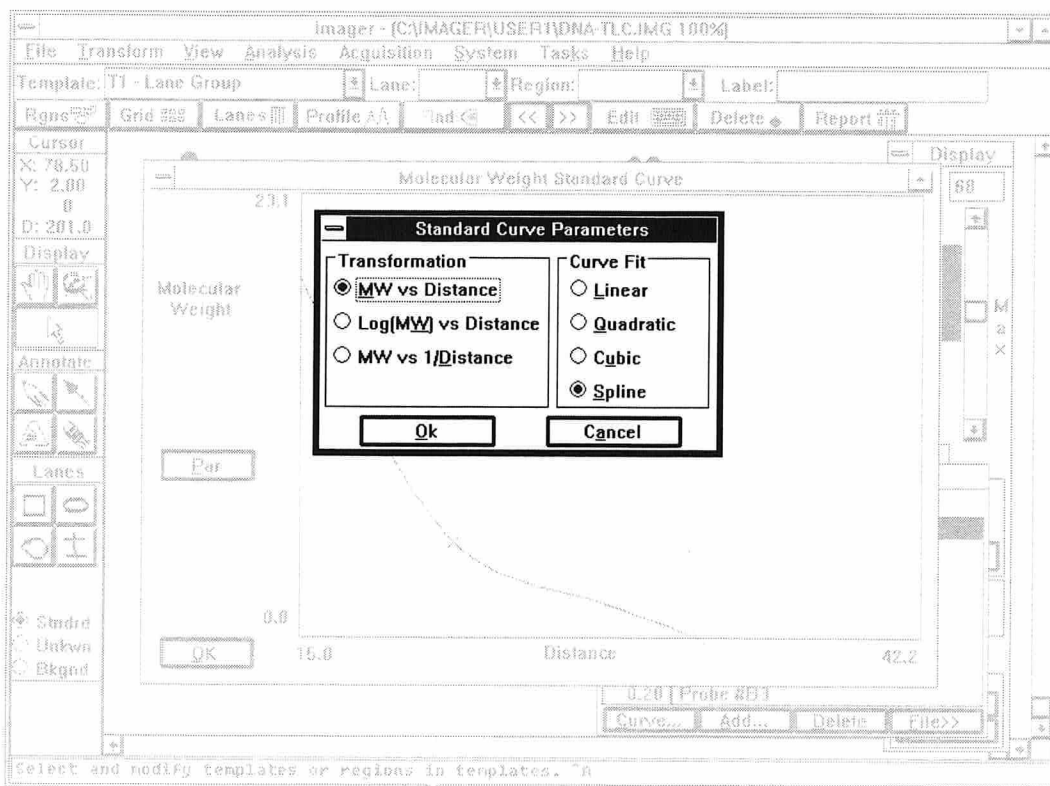


Figure 7-6. Standard Curve Parameters Window.

SELECTION**DESCRIPTION OF USE****Transformation:
MW vs Distance**

Takes the molecular weight and distance of each standard to plot the standard curve. This is the default axis transformation.

**Transformation:
Log [MW] vs Distance**

Takes the log of each molecular weight and the distance of each standard to plot the standard curve.

**Transformation:
MW vs 1/Distance**

Takes the molecular weight and the inverse of the distance of each standard to plot the standard curve.

Curve Fit: Linear

Uses the linear curve fitting method to draw the standard curve.

**Curve Fit:
Quadratic**

Uses the quadratic curve fitting method to draw the standard curve.

Curve Fit: Cubic

Uses the cubic curve fitting method to draw the standard curve.

Curve: Spline

Uses the spline curve fitting method to draw the standard curve. Spline is the default.

OK

Draws the standard curve based upon the transformation selection and curve fit selection.

CANCEL

Aborts the operation and closes the window.

Editing Lane Points

A lane point can be a region of interest (ROI) or a band marked with the Band Marking tool designated as an unknown, a background, or a standard. Lane points designated as standards have molecular weights associated with them.

You can change the type and molecular weight of a selected lane points using the **Edit Lane Points** window (Figure 7-7).

To display the **Edit Lane Points** window (a lane populated with lane points must be selected):

- ☐ Select **EDIT** from the template bar.

Or,

- ☐ Select **Edit Template** from the **Analysis** menu.

 **Note:** You can alternately double click on the tag of the lane.

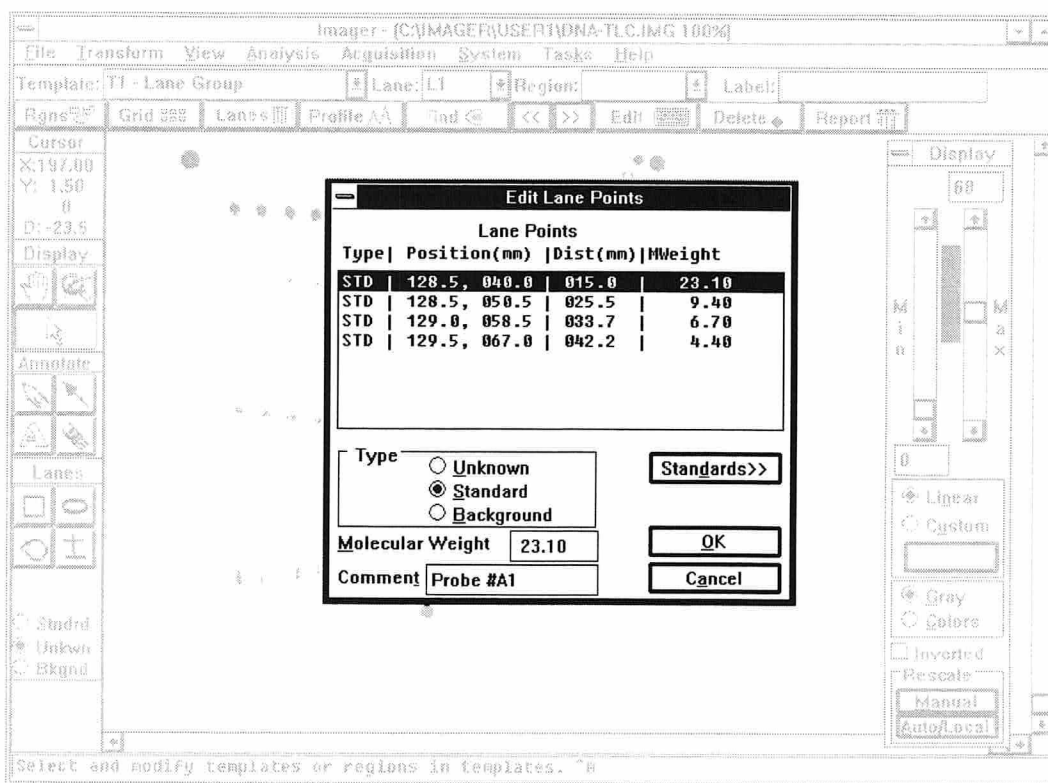


Figure 7-7. Edit Lane Points Window.

Changing Lane Point Types

You can change the type of each lane point in the **Editing Lane Points** window.

- ☐ Select a lane point in the list.
- ☐ Select a different lane point type ("Unknown", "Standard", or "Background").
- ☐ Select the lane point in the list again.

The lane point type is changed.

When you change a lane point from an unknown or a background to a standard, you should assign it a molecular weight. See *Changing Molecular Weights Individually* (page R266) or *Changing Molecular Weights Using the list* (page R267).

Changing Molecular Weights Individually

A molecular weight in the list can be changed by selecting the lane point in the list and entering a new value. The system automatically reorders the list when the value assigned to the molecular weight changes.

- ☐ Select a lane point in the list.
- ☐ Enter a new molecular weight at the "Molecular Weight" entry box.
- ☐ Select **OK**.

The new molecular weight is applied and the selected lane template is redisplayed.

Changing Molecular Weights Using the List

You can apply molecular weights to the lane points listed in the window from a list of predefined weights. Selecting **STANDARDS** displays the list of predefined standards (Figure 7-8). The molecular weights assigned to the lane points can be changed globally or selectively.

Global Change

To globally apply the weights listed on the right in the window to the ones listed to the left:

- ☐ Select **STANDARDS**.
- ☐ Select **COPY ALL**.

Selecting **COPY ALL** copies the list of molecular weight standards to the lane points. The first standard in the list is copied to the first point in the lane, the second standard in the list is copied to the second lane point in the list, etc.

Selective Change

To change the weights listed in the window selectively:

- ☐ Select **STANDARDS**.
- ☐ Select a lane point in the list (on the left).
- ☐ Select a molecular weight standard from the list (on the right).
- ☐ Select **COPY**.

Selecting **COPY** copies the selected molecular weight to the selected lane point.

Changing the Default List

Selecting **BROWSE** displays the **Select Molecular Weight Standards** window. This window enables you load an alternate list of molecular weight standards.

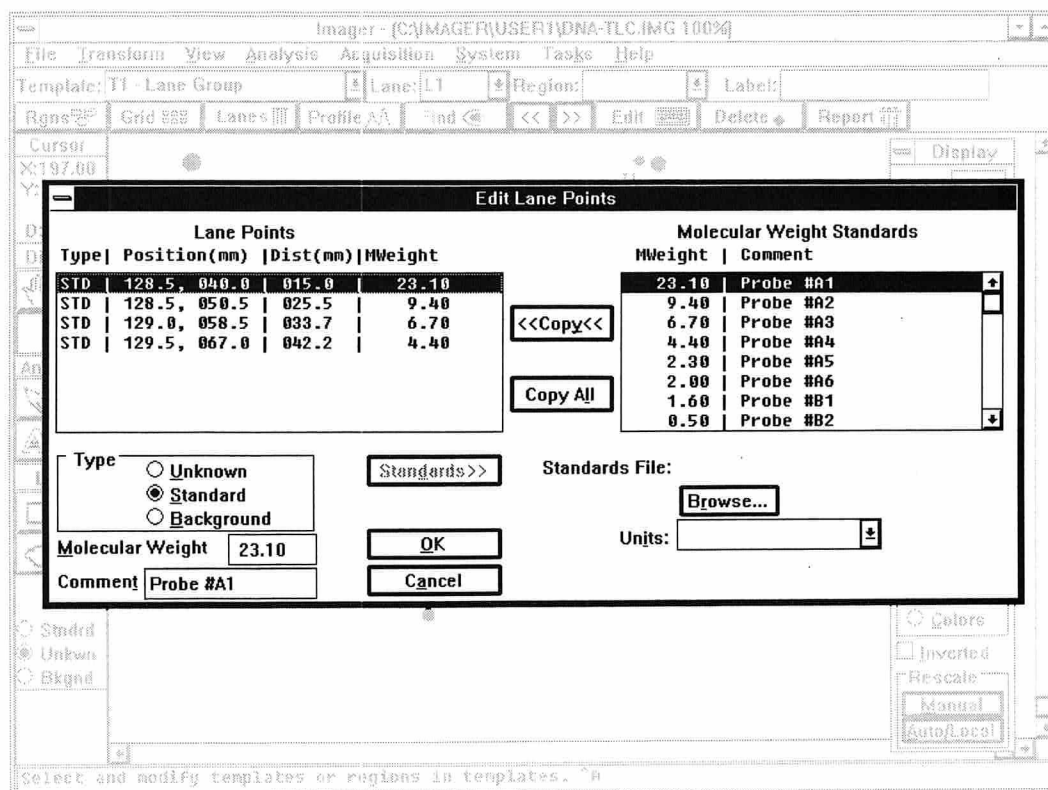


Figure 7-8. List of Standards at Edit Lane Points Window.

Reporting Lane Templates

See the Chapter *Reporting Results* for more information on reporting quantitative results obtained from lanes.